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14. ABSTRACT

Thus far we have recruited 36 breast cancer (BCa) patients of the planned total of 50 subjects. The women were stratified according to their circulating vitamin D status [25(OH)D] and treatment was dependent on their level of 25(OH)D. Of the 36 patients 15 did not receive treatment, 14 received 2000 IU of vitamin D, 5 received 4000 IU and 2 received 6000 IU. The dosing strategy changed during the trial, the current protocol based on circulating levels of vitamin D: > 40 ng/mL no treatment, 30-40 get 2000 IU, 20-30 get 4000 IU, <20 get 6000 IU. We have analyzed the biopsy samples (pre-intervention) and compared results with the surgical specimens (post-intervention) to determine whether dietary vitamin D supplements can regulate the gene expression profile in the BCa and normal cells. This has been completed in 20 subjects. The eventual analysis will determine whether vitamin D deficiency is associated with a poor prognosis profile and whether vitamin D intervention converts the profile toward a more normal profile of gene expression, perhaps representing a better prognosis.

15. SUBJECT TERMS
vitamin D, calcitriol, breast cancer, vitamin D receptor, estrogen receptor, antiproliferation, COX-2, prostaglandins, aromatase

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Introduction

Although the grant was supposed to start 9/1/07 the DOD human subjects approval process greatly delayed the start date. We finally received human subjects approval in mid-July and our Notice of Award was received on 3/13/08. We actually initiated work on the grant and began to charge effort to the grant on 4/1/08. We have requested and received a No Cost Extension to work on this project until June 30 2010, slightly over 2 full years which was the original time projected to complete the study.
The goals of the study are unchanged from the original grant. The recruitment of patients is well underway and the findings will be detailed below.

The work to be accomplished as described in the SOW.

1. To recruit patients undergoing core needle biopsy for breast abnormalities so as to accrue 50 evaluable patients with breast cancer to study.
2. To analyze their vitamin D, PTH, and calcium status
3. To characterize the classical prognostic and predictive characteristics of the breast cancer at time of diagnostic biopsy
4. To analyze the cancer biopsy specimens for a gene profile
5. To determine whether the prognostic and predictive factors differ between vitamin D deficient or insufficient patients and vitamin D sufficient patients.
6. To treat the vitamin D deficient and insufficient patients with vitamin D during the interval between biopsy and definitive breast surgery
7. To reanalyze the vitamin D status after intervention and before surgery
8. To analyze the surgical cancer specimens for their gene profile and compare the findings to the core biopsy gene profile to determine whether vitamin D therapy changes the profile
9. To compare the results of treated deficient/insufficient patients to sufficient patients to determine whether vitamin D intervention normalized the gene profile.
10. To analyze the data

Body

Recruitment of subjects with breast cancer is going well. At this time we have enrolled 36 patients. Their vitamin D [25(OH)D], $1,25(OH)_2D_3$ and other hormone levels as well as demographic data are shown in the accompanying table (Appendix). The patients have been stratified to receive vitamin D therapy depending on their initial 25(OH)D levels. The table also details the dose of vitamin D and the duration of therapy.

At this time we have measured the gene profiles of 20 of the subjects. The gene array and the data for these subjects is provided in tables in the Appendix. We are comparing the gene expression profile in the diagnostic biopsy with the surgical specimen obtained 2 to 5 weeks later depending on the interval until surgery takes place. We also are comparing normal regions in the surgery specimen to cancer regions.

A potential problem that had surfaced during the early days of the grant was the extremely small amount of tissue left for our study after the pathologist has completed the clinically relevant analysis of the diagnostic biopsy material. We have found that the yield of RNA from this residual amount of tissue was not sufficient to perform individual PCR analyses on the full complement of genes that we had planned to study. We therefore worked with a company (SABioscience) to tailor-make a gene array (described in the appendix) and to develop an RNA amplification strategy to generate enough RNA for the full analysis. This technique appears to be successful except that several low expressing genes are not being adequately detected. So although our array has 40 target genes plus 5 housekeeping genes and controls, we are obtaining useful data on fewer than 40 genes and only 4 housekeeping genes are being used in the analysis.

A second potential problem is that more of our subjects than expected from literature data were found to have adequate 25(OH)D levels and so they are not being treated with vitamin D. This means that we are finding fewer subjects that qualify to receive high doses of vitamin D intervention, even with the more aggressive strategy that we adopted when the protocol was changed. Currently 15 patients have no treatment, 14 have 2000 IU, 5 have 4000 IU and 2 received 6000 IU. Since surgery is generally scheduled very soon after the diagnosis is made, the treatment period is quite short. The combination of lower doses of vitamin D and shorter treatment times has resulted in somewhat smaller changes in the 25(OH)D levels than we had hoped to achieve. The delta between initial and final 25(OH)D levels and/or the ultimate level of 25(OH)D achieved are the likely parameters that will result in effective changes in gene transcription. Thus we are somewhat concerned to find that the dosage schedule we are using, drawn up years ago when concern about potential toxicity was greater, may have been structured too conservatively in order to avoid toxicity from too much vitamin D when in fact higher doses may have been more efficacious and still safe. We will have to wait until the gene expression data are fully analyzed but we are sorry that our concerns for the safety of our subjects, as well as IRB concerns, may have led us to be too conservative.

Key Research Accomplishments

Recruitment of 36 subjects to the study out of planned 50. Analysis of specimens from 20 of these subjects is completed. The remaining specimens are in the process of being assayed.

Reportable Outcomes

None yet

Conclusions

The recruitment of patients is going well. The gene expression analysis has been completed in 20 subjects. There are fewer vitamin D deficient patients than we expected based on published ratios of vitamin D levels in the population. Thus most subjects are not being treated (control group) and we are finding many women that get the low dose of vitamin D and many fewer that qualify for the middle and high dose. We anticipate that patient recruitment will be successful to end with 50 subjects. We have optimized the strategy to evaluate the profile of gene expression in the breast cancer biopsies and surgical specimens and these studies are moving forward.

References

N/A

Appendices

Table 1. Patient data showing recruited subjects including vitamin D levels and intervention.

Table 2. Pathology analysis of patient specimens.

Table 3. Description of the array and description of the genes on the array.

Table 4. Summary of some gene expression data from control and treated subjects.

TABLE 1: PATIENT DATA SHOWING RECRUITED SUBJECTS INCLUDING VITAMIN D LEVELS AND INTERVENTION

								Ref Ranges	**25-80 pg/ml**		**18-78 ng/ml**		**8.5-10.5 mg/dl**		**10-80 pg/ml**					
# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Surgery Status	Vitamin D intervention	Days betw draws
								Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery				
1	1	49	White/Non Hispanic	4/28/2008	Invasive Lobular Carcinoma	Pos	Neg	45	45	23	36	8.7	8.8	27	21	Mastectomy	Done	None	8
2	2	53	Asian/Non Hispanic	4/28/2008	Invasive Ductual Carcinoma	Neg	Neg	35	36	38	44	9.5	9.2	27	51	Lumpectomy	Done	None	16
3	3	45	White/Non Hispanic	4/29/2008	Invasive Ductual Carcinoma	Pos	Pos	26	36	75	62	9.2	9.1	30	31	Mastectomy	Done	One Capsule for 36 days	37
4	4	47	Other/ Hispanic	5/9/2008	Invasive Lobular Carcinoma	Awaiting results	Awaiting results	50	Not Done	58	Not Done	9.5	Not Done	11	Not Done	Mastectomy	Done	None	na
5	5	46	Asian/Non Hispanic	5/20/2008	Invasive Mixed ductal and micropapillary	Pos	Pos	14	26	36	57	9.5	9.5	31	33	Mastectomy	Done	Two Capsules for 12 days	14
6	6	35	White/Non Hispanic	6/5/2008	Invasive Ductual Carcinoma	Neg	Neg	33	35	98	45	9.7	9.5	5	21	Mastectomy	Done	None	34
7	7	47	Asian/Non Hispanic	6/26/2008	Invasive Ductual Carcinoma	Pos	Pos	27	35	45	48	9.1	9.6	20	18	Mastectomy	Done	One Capsule for 14 days	18
8	8	50	White/Non Hispanic	6/27/2008	Invasive Ductual Carcinoma	Pos	Pos	19	37	41	62	8.5	8.7	82	59	Lumpectomy	Done	One Capsule for 48 days	54
9	9	50	White/Non Hispanic	7/17/2008	Invasive Ductual and Tubular Carcinoma	Pos	Pos	39	41	63	71	9.8	8.9	25	29	Lumpectomy	Done	None	12

Vitamin D Study - Patient Log TABLE 1: CONTD

# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Surgery Status	Vitamin D intervention	Days betw draws
								Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery				
10	10	37	White/Non Hispanic	7/17/2008	Invasive Ductual, Tubular Carcinoma	Pos	Pos	38	26	60	56	9.7	7.9	36	25	Mastectomy	Done	None	32
11	11	57	White/Non Hispanic	7/29/2008	Invasive Ductual Carcinoma	Pos	Pos	32	29	47	47	10	9.5	76	45	Mastectomy	Done	None	42
12	12	71	White/Non Hispanic	7/30/2008	Invasive Mucinous Carcinoma	Pos	Pos	11	20	27	31	9.5	9.0	73	78	Lumpectomy	Done	Two Capsules for 15 days	19
13	13	54	White/Non Hispanic	8/7/2008	Invasive Ductual Carcinoma	L-pos R-Neg	L-pos R-Neg	49	38	62	58	9.1	9.3	31	32	Mastectomy	Done	None	46
14	14	56	Asian/Non Hispanic	8/26/2008	Invasive Ductual Carcinoma	Pos	Pos	51	47	44	47	9.4	9.3	42	43	Mastectomy	Done	None	16
15	15	40	Asian/Non Hispanic	10/6/2008	Invasive Ductual Carcinoma	Pos	Pos	22	27	48	51	9.6	9.6	27	26	Lumpectomy	Done	1 capsule for 15 days	16
16	16	55	White/Non Hispanic	11/17/2008	Invasive Ductual Carcinoma	Pos	Pos	14	21	60	61	9.3	9.0	70	75	Lumpectomy	Done	2 capsules for 6 days	8
17	18	63	White/Non Hispanic	3/26/2009	IDC and DCIS	Pos	Pos	17	26	27	29	9	8.5	38	50	Matectomy	done	1 capsule for 27 days	47
18	20	61	Other/ Hispanic	3/31/2009	Invasive Ductual Carcinoma	Pos	Pos	43	43	66	38	10.1	9.6	33	24	Mastectomy	done	None	56
19	22	51	White/Non Hispanic	5/1/2009	Invasive Ductual Carcinoma	Pos	Pos	23	30	48	44	9.1	8.6	30	25	**bilateral lumpectmy**	done	1 capsule for 30 days	38
20	23	39	Asian/Non Hispanic	5/15/2009	Invasive Ductual Carcinoma	Pos	Pos	17	28	60	77	8.5	9.1	72	21	Mastectomy	done	1 capsule for 12 days	14
21	24	51	White/Non Hispanic	5/22/2009	invasive Ductal Carcinoma	Pos	Neg	33	31	47	55	9.1	9.1	35	39	Mastectomy	done	None	18
22	25	56	White/Non Hispanic	6/1/2009	invasive Ductal Carcinoma	Pos	Pos	32	31	53	43	9.6	9.4	39	31	Bilat Mastectomy	done	None	17

* Blood drawn 7 days AFTER surgery.

Vitamin D Study - Patient Log TABLE 1: CONTD

# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Surgery Status	Vitamin D intervention	Days betw draws
								Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery				
23	26	65	White/Non Hispanic	6/4/2009	invasive Ductal Carcinoma	95%	95%	36	35	58	59	9.6	9.1	40	56	Lumpectomy	done 7/27	None	49
24	27	43		6/4/2008	Invasive Lobular Carcinoma	31%	22%	25	27	62	49	9.3	9	27	18	Lumpectomy	done	1 capsule for 12 days	18
25	28	63	White/Non Hispanic	6/24/2009	invasive Ductal Carcinoma	95,95%	50/90%	35	42	62	70	9.1	9.4	25	18	Bilat Mastectomy	done 7/29	1 capsule for 34 days	50
26	33	60	White/Non Hispanic	7/10/2009	invasive Ductal Carcinoma	100%	90%	33	29*	42	na	8.9	8.6	46	16*	Bilat Mastectomy	done 8/7	1 capsule for 20 days	na
27	35	71	White/Non Hispanic	7/21/2009	invasive Ductal Carcinoma			33	37	68	75	8.6	8.8	70	32	Mastectomy	done 9/1	1 capsule 35 days	41
28	38	46	Other/ Hispanic	7/31/2009	invasive Ductal Carcinoma	90%	95%	17	48	82	56	9.1	9.3	94	84	Lumpectomy		3 capsules for 50 days	60
29	40	50	Pacific Islander	8/14/2009	Invasive Ductal Carcinoma	90%	80%	19	42	58	48	8.9	9	41	38	Mastectomy	2-Nov	3 capsules for 73 days	79
30	41	27	White/Non Hispanic	8/18/2009	Invasive Ductal Carcinoma			40	39	58	36	9	9.2	35	39	Bilat Mastectomy	11-Sep	1 capsule for 15 days	23
31	42	59	White/Non Hispanic	8/27/2009	ID C			27	45	63	89	9.4	9.3	45	32	Lumpectomy	28-Sep	2 capsules for 33 days	39
32	44	63	White/Non Hispanic	10/21/2009	IDC 41					65		9.2		16			5-Jan	none	
33	45	54	Asian/Non Hispanic	10/30/09	IDC	>95%	neg	30	39	47	59	9.7	8-Sep	26	27	PAMF pt	3-Dec	2 capsules for 30 days	32
34	46	73	Asian/Non Hispanic	11/5/09	IDC and DCIS	3+	3+	39		37		10		19			6-Jan	1 capsule	
35	47	89	White/Non Hispanic	11/12/09	IDC			34		26		10.5		113					
36	48	65	White/Non Hispanic	11/13/09	IDC	3+	3+	39		68		9	8.7	44		Mills pt	18-Dec	1 capsule for 30 days	33

* Blood drawn 7 days AFTER surgery.

TABLE 2: PATHOLOGY ANALYSIS OF PATIENT SPECIMENS

ID number	Age	Out_core?	Out_res?	Tumor_side	Tumor_site	Histology[1]	Grade[1]	Size[1]	Nodes
1	49	Yes	No	Left	2 o'clock	Lobular	2	4.8 cm[2]	0/4
2	53	No	No	Left	3:30	Ductal	3	2.4 cm	0/3
3-A (1)	45	Yes	No	Right	11:00-12:00	Ductal	2	2.4 cm	1/4
3-B (2)	45	Yes	No	Right	Central	Ductal	1	2.5 cm	1/4
4	47	Yes	Yes	Right	Unknown	Lobular	1-2	6 cm	1/4
5	45	No	No	Right	UOQ	Ductal and micropapillary	2	3.9 cm	2/21
6	35	Yes	No	Right	11:30	Ductal	3	1.5 cm	1/15
7	50	Yes	No	Right	9:30	Ductal	2	2.3 cm	0/5
8	47	Yes	No	Left	Subareolar	Ductal	3	3.5 cm	2/29
9	50	Yes	No	Left	11:00	Ductal	1	0.4 cm	0/2
10	36	Yes	No	Right	12:00	Ductal	3	3.5 cm	0/12
11-A (1)[3]	57	No	No	Right	3:00	Ductal	1	2.1 cm	0/4
11-B (2)	57	No	No	Right	6:00	Ductal	1	0.4 cm	0/4
11-C (3)	57	No	No	Right	6:00	Ductal	1	0.6 cm	0/4
12	71	No	No	Left	retroareolar	Ductal with mucinous features	1	1.9 cm	0/1[4]
13-A (1)	54	Yes	No	Left	1:00	Ductal	2	0.7 cm	0/3
13-B (2)	54	Yes	No	Left	10:00	Ductal	2	1.8 cm	0/3
13-C (3)[5]	54	No	No	Left	Lateral lower quadrant	Lobular	1	0.9 cm	0/3
14	56	Yes	No	Left	10:00-12:00	Ductal	1	3.5 cm	0/4
15	40	Yes	No	Right	2:00 subareolar	Ductal	3	1.8 cm	3/20
16[6]	55	Yes	No	Left	12:00	Ductal	2	1.2 cm	0/16
18	62	Yes	No	Left	3:30-4:00	Ductal	3	5.4 cm	1/10
20	62	Yes	No	Right	7:00	Ductal	2	0.4 cm[7]	1/4
22-A (Lt)	51	No	No	Left	6:00	Ductal	1	0.7 cm	0/3
22-B (Rt)	51	No	No	Right	9:30	Ductal	3	2.5 cm[8]	0/4
23	39	Yes	No	Left	2:00	Ductal	3	2.8 cm	1/8
24	51	Yes	No	Right	UOQ	Ductal with metaplastic differentiation	3	2.7 cm[9]	0/8
25	56	Yes	No	Right	11:00	Ductal	2	1.2 cm	0/4[4]
26	65	Yes	No	Right	Lower breast	Ductal	1	1.5 cm	0/5
27	43	No	No	Left	LOQ	Lobular	1	5.7 cm	2/25[10]
28-A (L)[11]	63	Yes	No	Left	Not known	Ductal	1	Not known	0/4
28-A (R)[11]	63	Yes	No	Right	11:00	Ductal	2	Not known	0/3
33	60	Yes	No	Right	9:00	Ductal	1	0.9 cm[12]	0/4
35	71	No	No	Right	5:00	Ductal	2	1.2 cm	0/1
38	46	Yes	No	Left					
40	50	Yes	No	Left	3:00	Ductal			
41	27	Yes	No	Left	3:00	Ductal			
42	59	Yes	No	Right	10:00	Ductal			

[1]Histology, grade, size taken from resection unless otherwise specified

[2]Size garnered from addition of two lumpectomies and subsequent completion mastectomy

[3]Only tumor one tested by Feldman lab with matching core/resection pair

[4]Isolated (less than 10) keratin-positive cells on keratin stain only

[5]Not tested by Feldman lab

[6]To date (12/15/09), outside core needle biopsy block cannot be found

[7]Four separate foci ranging from 0.2 cm to 0.4 cm

[8]Smaller focus 0.9 cm, not tested by Feldman lab

[9]Smaller focus 0.9 cm, not tested by Feldman lab

[10]Additional 6 lymph nodes showed keratin-positive cells

[11]No residual infiltrating cancer at resection in either breast

[12]Multifocal, size is largest focus

ID number	D numbe	PathTNM	In situ present?	Stage	Core_ER_int	Core_ER_pct	Core_ER_Allred	Core_PR_int	Core_PR_pct	Core_PR_Allred	Core_Ki67_pct	Core_HER2
1	1	pT2pN0(i-)pMX	No									
2	2	pT2pN0(i-)pMX	No		0	0	0	0	0	0	80	
3-A (1)	3-A (1)	same as above	No		2	95	7	3	95	8	10	
3-B (2)	3-B (2)	pT2pN1mi(sn)pMX	Yes (DCIS)									
4	4	pT3pN1mi(sn)pMX(?)	Yes (LCIS)									
5	5	pT2pN1pMX	Yes (DCIS)		2	90	7	3	95	8	30	1.17
6	6	pT1cpN1mipMX	Yes (DCIS)									
7	7	pT2pN0(i-)pMX	Yes (DCIS)									
8	8	pT2pN1apMX	Yes (DCIS)									
9	9	pT1apN0(i-)pMX	No									
10	10	pT2pN0(i-)pMX	Yes (DCIS)									
11-A (1)[3]	11-A (1)[3]	pT2pN0(i-)pMX	Yes (DCIS)									
11-B (2)	11-B (2)	same as above	No		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11-C (3)	11-C (3)	same as above	Yes (DCIS)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
12	12	pT1cpN0(i+)pMX	No									
13-A (1)	13-A (1)	pT1cpN0(i-)pMX	Yes (DCIS)									
13-B (2)	13-B (2)	same as above	Yes (DCIS)									
13-C (3)[5]	13-C (3)[5]	same as above	Yes (LCIS)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
14	14	pT2pN0pMX	Yes (DCIS)									
15	15	pT1cpN1apMX	Yes (DCIS)									
16[6]	16[6]	pT1cpN0(i-)pMX	Yes (DCIS)									
18	18	pT3pN1pMX	Yes (DCIS)		3	100	8	2	5	4	85	0.53
20	20	pT1apN1mipMX	No									
22-A (Lt)	22-A (Lt)	pT1bpN0(i-)pMX	Yes (DCIS)		3	95	8	3	95	8	5-10	1.26
22-B (Rt)	22-B (Rt)	pT2pN0(i-)pMX	Yes (DCIS)									
23	23	pT2pN1mipMX	Yes (DCIS)									
24	24	pT2pN0(i-)pMX	Yes (DCIS)									
25	25	pT1cpN0(i+)pMX	Yes (DCIS)									
26	26	pT1cpN0(i-)pMX	Yes (DCIS)									
27	27	pT3pN1apMX	Yes (LCIS)									
28-A (L)[11]	28-A (L)[11]	pTXpN0(i-)pMX	Yes (DCIS)									
28-A (R)[11]	28-A (R)[11]	pTXpN0(i-)pMX	Yes (DCIS)									
33	33	pT1bpN0(i-)pMX	Yes (DCIS)									
35	35	pT1cpN0(i-)pMX	Yes (DCIS)									
38	38											
40	40											
41	41											
42	42											

				TABLE 2: CONTD					
ID number	ID number	Res_ER_int	Res_ER_pct	Res_ER_Allred	Res_PR_int	Res_PR_pct	Res_PR_Allred	Res_Ki67_pct	Res_HER2
1	1	3	95	8	3	95	8	20	0.98
2	2	0	0	0	0	0	0	80	1.07
3-A (1)	3-A (1)	2	50	6	3	50	7	20	1.02
3-B (2)	3-B (2)	2	80	7	3	95	8	10	1.44
4	4								
5	5								
6	6	0	0	0	0	0	0	35	1.07
7	7								
8	8	2	95	7	3	15	6	15	1.38
9	9								
10	10								
11-A (1)[3]	11-A (1)[3]	3	95	8	2	10	4	<5	1.07
11-B (2)	11-B (2)	3	95	8	3	30	6	<5	1.09
11-C (3)	11-C (3)	3	95	8	3	95	8	<5	1.05
12	12	2	95	7	3	95	8	10	1.05
13-A (1)	13-A (1)	2	80	7	3	95	8	<5	0 (IHC)
13-B (2)	13-B (2)	2	95	7	3	95	8	<5	0 (IHC)
13-C (3)[5]	13-C (3)[5]	2	20	5	3	95	8	<5	0 (IHC)
14	14								0.97
15	15	2	80	7	3	80	8	<5	3.42
16[6]	16[6]	3	95	8	2-3	90	7-8	5-10	0.94
18	18								
20	20								
22-A (Lt)	22-A (Lt)								
22-B (Rt)	22-B (Rt)	3	95	8	3	95	8	70	1
23	23	1-2	50	5-6	2-3	70	7-8	20	0.84
24	24								
25	25	3	95	8	3	<1%	4	5	0.98
26	26	3	95	8	3	95	8	<5	0.98
27	27								
28-A (L)[11]	28-A (L)[11]								
28-A (R)[11]	28-A (R)[11]								
33	33	3	95	8	3	70	8	0	1.37
35	35	3	95	8	0	0	0	10	1.55
38	38								
40	40								
41	41								
42	42								

TABLE 3: DESCRIPTION OF THE ARRAY AND GENES IN THE ARRAY

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTB	GAPDH	RPLP0	GUSB	TFRC	ESR1	ESR2	PGR	CYP19A1	TFF1	ERBB2	GRB7
B	EGFR	MKI67	AURKA	BIRC5	CCND1	CCNA1	TP53	MMP11	CTSL2	BAX	BCL2	VDR
C	CYP24A1	CYP27B1	HR	SNAI2	MYC	PTGS2	HPGD	PTGER4	DUSP10	IL6	TGFB1	TNF
D	CDKN1A	IGFBP3	SPP1	AR	PTHLH	AMH	FABP5	PPARG	GSTM1	GDC	RTC	PPC
E	ACTB	GAPDH	RPLP0	GUSB	TFRC	ESR1	ESR2	PGR	CYP19A1	TFF1	ERBB2	GRB7
F	EGFR	MKI67	AURKA	BIRC5	CCND1	CCNA1	TP53	MMP11	CTSL2	BAX	BCL2	VDR
G	CYP24A1	CYP27B1	HR	SNAI2	MYC	PTGS2	HPGD	PTGER4	DUSP10	IL6	TGFB1	TNF
H	CDKN1A	IGFBP3	SPP1	AR	PTHLH	AMH	FABP5	PPARG	GSTM1	GDC	RTC	PPC

GDC: Genomic DNA contamination control

RTC: Reverse transcription control

PPC: Positive PCR control

TABLE 3 CONTD.

PCR Array Catalog #:		Feldman		
Position	**UniGene**	**RefSeq**	**Symbol**	**Description**
A01	Hs.520640	NM_001101	ACTB	Actin, beta
A02	Hs.544577	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
A03	Hs.546285	NM_001002	RPLP0	Ribosomal protein, large, P0
A04	Hs.255230	NM_000181	GUSB	Glucuronidase, beta
A05	Hs.529618	NM_003234	TFRC	Transferrin receptor (p90, CD71)
A06	Hs.208124	NM_000125	ESR1	Estrogen receptor 1
A07	Hs.443150	NM_001437	ESR2	Estrogen receptor 2 (ER beta)
A08	Hs.32405	NM_000926	PGR	Progesterone receptor
A09	Hs.654384	NM_000103	CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1
A10	Hs.162807	NM_003225	TFF1	Trefoil factor 1
A11	Hs.446352	NM_004448	ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
A12	Hs.86859	NM_005310	GRB7	Growth factor receptor-bound protein 7
B01	Hs.488293	NM_005228	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
B02	Hs.80976	NM_002417	MKI67	Antigen identified by monoclonal antibody Ki-67
B03	Hs.250822	NM_003600	AURKA	Aurora kinase A
B04	Hs.514527	NM_001168	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)
B05	Hs.523852	NM_053056	CCND1	Cyclin D1
B06	Hs.417050	NM_003914	CCNA1	Cyclin A1
B07	Hs.654481	NM_000546	TP53	Tumor protein p53
B08	Hs.143751	NM_005940	MMP11	Matrix metallopeptidase 11 (stromelysin 3)
B09	Hs.660866	NM_001333	CTSL2	Cathepsin L2
B10	Hs.631546	NM_004324	BAX	BCL2-associated X protein
B11	Hs.150749	NM_000633	BCL2	B-cell CLL/lymphoma 2
B12	Hs.524368	NM_000376	VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor
C01	Hs.89663	NM_000782	CYP24A1	Cytochrome P450, family 24, subfamily A, polypeptide 1
C02	Hs.524528	NM_000785	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1
C03	Hs.272367	NM_018411	HR	Hairless homolog (mouse)
C04	Hs.360174	NM_003068	SNAI2	Snail homolog 2 (Drosophila)
C05	Hs.202453	NM_002467	MYC	V-myc myelocytomatosis viral oncogene homolog (avian)
C06	Hs.196384	NM_000963	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
C07	Hs.655491	NM_000860	HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)
C08	Hs.199248	NM_000958	PTGER4	Prostaglandin E receptor 4 (subtype EP4)
C09	Hs.497822	NM_007207	DUSP10	Dual specificity phosphatase 10
C10	Hs.654458	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
C11	Hs.645227	NM_000660	TGFB1	Transforming growth factor, beta 1
C12	Hs.241570	NM_000594	TNF	Tumor necrosis factor (TNF superfamily, member 2)
D01	Hs.370771	NM_000389	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
D02	Hs.450230	NM_000598	IGFBP3	Insulin-like growth factor binding protein 3
D03	Hs.313	NM_000582	SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
D04	Hs.496240	NM_000044	AR	Androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)
D05	Hs.591159	NM_002820	PTHLH	Parathyroid hormone-like hormone
D06	Hs.112432	NM_000479	AMH	Anti-Mullerian hormone
D07	Hs.408061	NM_001444	FABP5	Fatty acid binding protein 5 (psoriasis-associated)
D08	Hs.162646	NM_015869	PPARG	Peroxisome proliferator-activated receptor gamma
D09	Hs.301961	NM_000561	GSTM1	Glutathione S-transferase M1
D10	N/A	SA_00105	HGDC	Human Genomic DNA Contamination
D11	N/A	SA_00104	RTC	Reverse Transcription Control
D12	N/A	SA_00103	PPC	Positive PCR Control

TABLE 4: SUMMARY OF SOME GENE EXPRESSION DATA FROM CONTROL AND TREATED SUBJECTS

Surgery Cancer Region vs Surgery Normal Region

Patient	ER +/-	25-D	1,25-D	Expression Levels of Genes													
ID		Pre/Post	Pre/Post	p21	IGFBP-3	15-PGDH	TGFβ	TNFα	Ki67	ARUKA	BIRC5	MPM11	CTSL2	BAX	BCL2	ERα	ERβ
1	+	45/45	23/36	1	0.8	0.1	2.4	0.5	11	2.1	1.2	19	0.7	1.3	1.3	2.1	0.6
2	-	35/36	38/44	0.2	0.2	0.3	0.4	0.6	3.2	2	7.5	4.8	4.5	0.7	0.3	0.2	0.5
9	+	39/41	63/71	0.5	0.3	0.8	1.1	3.7	2.1	12	30	1.3	0.3	1.6	0.9	0.3	3
10	+	38/26	60/56	0.3	0.4	0.3	1.2	1.5	2.4	0.5	1.5	6.3	0.2	0.9	0.4	1	0.3
11	+	32/29	47/47	3.3	0.4	0.8	0.3	19	0.6	2.1	8.7	1.7	11	0.7	0.1	1.1	0.6
6	-	33/35	98/45	0.1	0.6	0.5	1.9	0.9	3.5	0.6	0.8	40	0.1	1.2	1.4	2	0.7
14	+	51/47	44/47	1.2	0.9	0.5	0.6	0.3	0.7	0.7	0.8	48	11	0.7	0.7	11	0.3
26	?	36/35	58/59	0.2	0.5	0.9	0.7	1.9	4.8	1.9	3.5	80	2.7	1.3	1.6	7.2	0.9
Average				0.9	0.5	0.5	1.1	3.6	3.5	2.7	6.8	25	3.8	1.1	0.8	3.1	0.9

Post vs Pre

Patient	ER	Dose	Treat	25-D		1,25-D	Expression Levels of Genes												
ID		25-D	(Days)	Pre/Post	Δ-D25	Pre/Post	p21	IGFBP-3	15-PGDH	TGFβ	TNFα	Ki67	ARUKA	BIRC5	MMP11	CTSL2	BAX	BCL2	ERα	ERβ
3	+	1	36	26/36	10	75/62	2.1	1.8	1	1.1	0.7	0.95	1.7	1.3	0.3	4.5	1.2	1.1	0.25	3
7	+	1	14	27/35	8	45/48	1.9	1.9	2.4	0.9	0.6	1.4	1.1	3.1	1	1.7	1.3	1	1.5	0.5
15	+	1	15	22/27	5	48/51	3.2	6.7	17	2.2	1.9	0.5	1.1	1.8	0.1	3	1.3	3.2	1.1	1.8
22	+	1	30	23/30	7	48/44	2	2.5	3.2	2.2	0.7	1	1	1	2.2	0.9	1.2	0.8	0.8	0.7
23	+	1	12	17/28	11	60/77	2	2.3	2.2	1.2	0.6	0.7	0.9	0.9	2.2	0.9	1.3	1.2	1.2	1
35	?	1	35	33/37	4	68/75	0.6	4.9	2.1	1.6	0.2	0.7	1	0.4	0.2	0.9	1.1	0.8	0.4	1.8
38	+	3	50	17/48	31	82/56	7.4	5.9	8.1	4	3.9	1.7	3	5.5	1	11.3	2.7	1.5	1.2	2.3
41	?	1	15	40/39	-1	58-?	3	2.8	6.2	0.8	0.9	1.3	0.9	0.7	0.2	1.4	0.9	0.6	0.4	1.8
42	?	2	33	27/45	18	63/?	6.8	2.1	4	1.2	1.7	0.9	1.6	1.2	1.1	0.9	1.7	0.9	0.9	1.3
Average							3.2	3.4	5.1	1.7	1.2	1.0	1.4	1.8	0.9	2.8	1.4	1.2	0.9	1.6